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A FLOW-THROUGH SYSTEM FOR EXPOSURE OF SEAGRASS TO POLLUTANTS*

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ABSTRACT

A flow-through system for exposure of seagrass to pollutants is described. Sea water with dissolved atrazine or PCP (pentachlorophenol) was pumped through a sealed 2-litre volume glass exposure vessel that contained either whole plants or leaves of Thalassia testudinum. *Effects of the compounds on oxygen evolution and uptake by leaves were measured after exposure for 40 and 88 h. Rate of oxygen evolution was depressed strongly after 40 h by 1 ppm of atrazine or PCP. The rate of oxygen uptake was slightly depressed by atrazine but strongly depressed by PCP. Photosynthesis/respiration ratios were depressed to less than one by 1 ppm of atrazine or PCP and by 0·5 ppm atrazine. EC_{50} values based upon depression of oxygen evolution after 40 hours' exposure were: atrazine, 0·32 ppm; PCP, 0·74 ppm. The data suggest that leaves may recover from, or adapt to, the presence of either toxicant.*

INTRODUCTION

Seagrass occurs in dense beds in shallow-water coastal environments of tropical and temperate oceans. The group consists of 49 angiosperm species (den Hartog, 1970) that reproduce either sexually by flowering, or vegetatively from rhizomes. Ecosystems dominated by seagrass are highly productive and a dense stand may consist of 4000 plants per square metre with a standing stock of 2 kg dry weight per square metre (McRoy & Helfferich, 1977). Buesa (1977) reported that *Thalassia testudinum*, a common seagrass of the Caribbean Sea, fixed between 11 and 35 times more carbon than phytoplankton in some coastal areas.

* Contribution No. 426 from the Gulf Breeze Laboratory.

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Within recent years, there has been a decline in the number and density of seagrass beds in many parts of the world. For example, decreases in abundance have been reported from the United States (McNulty, 1961), Japan (Kikuchi & Peres, 1977) and Australia (Larkum, 1977). In each case, decline was attributed to pollution.

We report here a method for exposing *T. testudinum* to pollutants in a flow-through system. In previous studies (Walsh & Garnas, 1982), death, apparent degradation, and survival were used to estimate effects of pollutants. Such studies took up to 4 weeks to complete and were imprecise, depending upon the subjective judgment of individual observers. The new method requires only 40 h of exposure and uses rates of dissolved oxygen flux to measure effects precisely.

METHODS

Thalassia testudinum was collected from shallow water in Santa Rosa Sound, Florida (approximately 87°9′ west longitude, 30°20′ north latitude) with a shovel. Healthy plants were removed from the centre of the clod, washed with sea water and taken immediately to the laboratory.

The exposure system (Fig. 1) was kept in a controlled environment room at 20°C with light from fluorescent tubes on a 12-h on–off cycle. Photosynthetically active radiation (PAR, 400–700 nm waveband) was measured with an LI-550 printing integrator fitted with an LI-192S underwater quantum sensor (Lambda Instruments Corp., Lincoln, Nebraska, USA). Intensity of PAR just below the acrylic plastic lid of the exposure vessel was 160 μE m^{-2} s^{-1}.

The toxicants tested were technical grade atrazine (99·7% pure) and pentachlorophenol (PCP, 98% pure). Stock solutions were prepared in 50% nanograde acetone and deionised water and diluted with 50% acetone to yield nominal concentrations of 0·01, 0·1, 0·5 and 1 parts per million (ppm) in exposure kettles. In one test, concentrations of atrazine were measured with a nitrogen–phosphorus detector on a gas chromatograph:

Atrazine (Parts per million)

Nominal	*Measured*
0·010	0·017
0·50	0·62
1·0	1·1

Exposure medium was artificial seawater prepared by dissolving a commercial sea salt mix (Rila Products, Teaneck, NJ, USA) in deionised water to a salinity of 30 parts per thousand. Medium was not filtered, nutrients were not added and sterile procedures were not used.

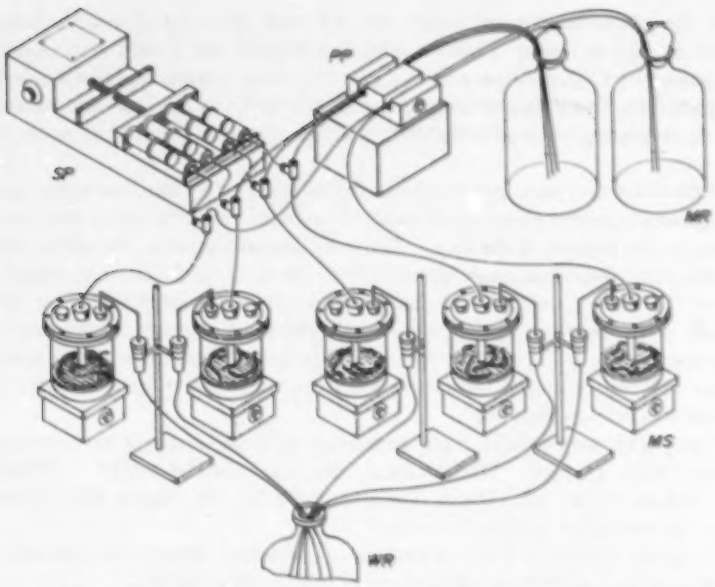

Fig. 1. Flow-through system for exposure of seagrass to pollutants. Screen for protection of leaves from the stirring bar is shown. MR—medium reservoir, MS—magnetic stirrer, PP—peristaltic pump, SP—syringe pump, WR—waste reservoir.

Medium was stored in covered linear polyethylene carboys and pumped directly to the exposure tanks by peristaltic pumps. It was carried to the pump in Tygon® (Norton Co., Akron, OH, USA) tubing of 4·8 mm inside diameter. The tubing was connected by a short length of glass tubing (6 mm OD) to Silastic® (Dow Corning, Midland, MI, USA) tubing (3·18 mm ID) which passed through the pump. Silastic tubing was used because it resists breaking by the continual kneading action of the pump.

On the delivery side of the pump, the Silastic tubing was attached to a T-tube connected by Tygon tubing to an infusion/withdrawal pump that held four 10-ml syringes, one with 50% nanograde acetone in deionised water and three with different concentrations of test chemicals in 50% acetone. This concentration of acetone was used to avoid precipitation when the acetone solution mixed with medium in the T-tube. Rate of flow from each syringe was 0·092 ml h^{-1}.

Tygon tubing carried the medium–toxicant–acetone mixture into the exposure

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vessel. An untreated control vessel received medium pumped directly from the carboy through a Tygon–Silastic tubing line without the T-tube attachment.

Exposure of *T. testudinum* was in a two-litre volume glass reaction kettle fitted with an acrylic plastic lid and neoprene gasket to make the seal air- and watertight. Lid and gasket were secured to the kettle with seven stainless steel bolts, washers and wing nuts.

The lid had three holes: one in the centre was fitted with a size 2 neoprene stopper through which passed a 6 mm glass tube that extended approximately two-thirds of the way to the bottom of the kettle. This tube was connected to the peristaltic and infusion/withdrawal pumps. A second hole contained a size 2 neoprene stopper and a 6 mm OD glass U-tube that drained medium from the interface of the lid and medium. This overflow dripped into a disposable plastic pipette tip connected to a collecting carboy by Tygon tubing. The third hole contained a size 3 neoprene stopper that was replaced by an oxygen electrode when oxygen content of the medium was being measured.

Oxygen and temperature were measured with an Orbisphere Laboratories Multichannel Oxygen Measurement System, Model 2710 (Orbisphere Laboratories, York, ME, USA). The five probes in this system were calibrated against air-saturated artificial sea water.

A magnetic stirring bar in the bottom of the kettle, which was mounted on a magnetic stirrer, stirred the medium constantly during the test.

The rate of flow of medium was 100 ± 10 ml h^{-1}. The rate of flow of acetone and pesticide into the connecting T-tube was adjusted according to final concentrations desired in the kettle.

Either whole plants or leaves were exposed to the toxicants. Ten whole plants were planted in sand in an acrylic plastic container with a false bottom (Fig. 2). They were prepared by careful collection and washing in clean sea water. Dead leaves were removed and the rhizome cut with a sharp razor blade 15 mm on either side of the stem. Each plant was blotted dry, weighed and carefully planted in sand that filled the container to approximately 1 cm from the top. Water flow from the stirring bar was upward along the wall of the kettle and down through the hole in the centre. *Thalassia* remained healthy for up to 28 days in this system.

Only green leaves were used in the second type of exposure. They were cut from the plant, rinsed in clean sea water, blotted dry and weighed. Ten $\pm 0{\cdot}1$ g were placed in each kettle. An acrylic plastic ring, 11 cm in diameter and covered with a linear polyethylene screen of 1 mm mesh, was placed in the kettle where the sides curve vertically inward to keep the leaves from contact with the stirrer. The leaves remained healthy for up to 2 weeks in this system.

The test consisted of two phases: an equilibration phase of approximately 72 h that provided baseline data on oxygen evolution by the seagrass in the absence of toxicant and an 88-h exposure phase in which the seagrass was exposed to the toxicant.

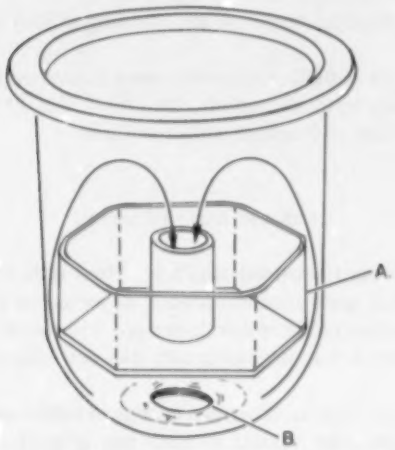

Fig. 2. Reaction kettle with acrylic plastic container (A) for exposure of seagrass to toxicants. Water flow, caused by action of the stirring bar (B), is upward along kettle walls and downward through the hole in the container.

In a typical test, *T. testudinum* was collected on the morning of Day 1 and leaves added to each of five previously prepared kettles. The kettles were filled with medium and sealed, eliminating all bubbles under the lid. The peristaltic pump was turned on and the flow rate adjusted to $100 \pm 10\,ml\,h^{-1}$. The infusion/withdrawal pump was not turned on. The system was allowed to stabilise at 20 °C until about 1600 h on Day 3 when the pump and lights were turned off.

On the morning of Day 4, the size 3 stoppers were removed and calibrated oxygen/temperature probes were carefully set into each kettle so as not to introduce air bubbles into the system. Dissolved oxygen and temperature were measured in each kettle. A small flashlight provided light for reading the meter in the dark room. The lights were then turned on and oxygen and temperature recorded at 30-min intervals for 4 h. The lights were turned off and oxygen and temperature again measured at 30-min intervals for 4 h.

At the end of this 8-h pretreatment test, the light and both pumps were turned on. One kettle was kept as an untreated control, one was the 50% acetone control and three were treated with test chemical in 50% acetone. To obtain the desired concentration of toxicant immediately in each kettle, 1 ml of each toxicant in 50% acetone was added through the hole that held the oxygen probe. One millilitre of 50% acetone was also added to the acetone-control kettle. The size 3 stopper was placed in the hole and toxicant in acetone supplied by the infusion/withdrawal pump maintained the nominal concentration.

After 24 h (pm, Day 5), the pumps and lights were again turned off and a test of

oxygen evolution and uptake began on the morning of Day 6, 40 h after initiation of exposure.

After the test on Day 6, lights and pumps were turned on until about 1600 h on Day 7, when they were again turned off. On Day 8, am, 88 h after exposure was begun, oxygen evolution and uptake were measured.

RESULTS AND DISCUSSION

Leaves and whole plants responded similarly. Most tests were done with leaves because they permitted more accurate control of weight of photosynthetic tissue. Data on single tests reported here were obtained with leaves.

Temperature variation was negligible and did not influence oxygen measurements.

Rates of evolution and uptake of oxygen in control kettles were similar before and after treatment of the other kettles in each test (Fig. 3). The ratio of rate of photosynthesis to rate of respiration (P/R) was always above 1·2 in untreated control and acetone-treated control kettles, suggesting that metabolism was not affected adversely by the exposure system. There was also very slight statistical variation within and between tests on each kettle, but rates of oxygen flux varied from kettle to kettle (Figs 4 and 5). Variation between kettles was not considered significant in analyses of the effects of pollutants because comparison of oxygen flux

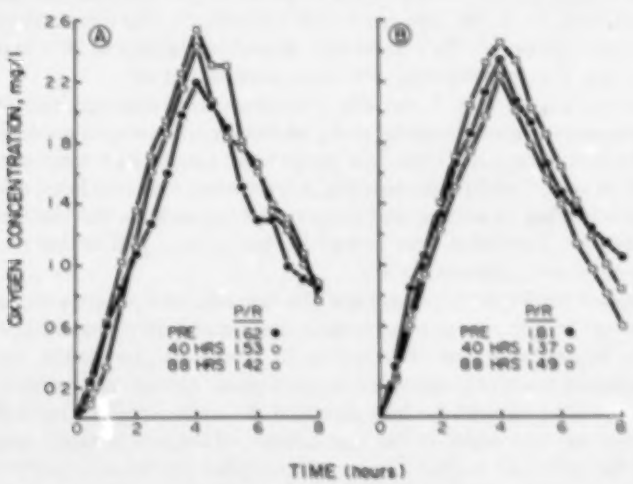

Fig. 3. Evolution and uptake of oxygen by *T. testudinum* in control systems. A—seawater control; B—acetone control; P/R—ratio of rate of photosynthesis to rate of respiration.

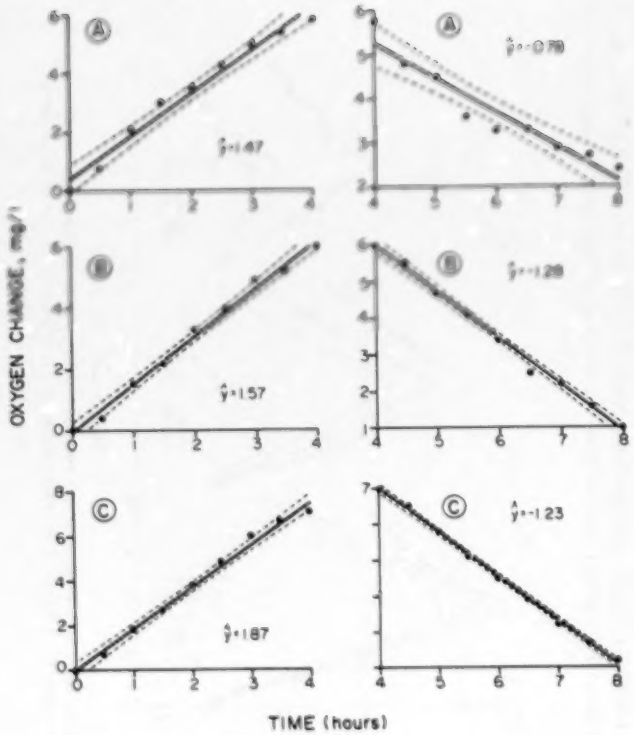

Fig. 4. Linear regression of oxygen evolution and uptake on time by *T. testudinum* in seawater control. A—pretreatment; B—40 h after treatment; C—88 h after treatment. Dashed lines represent the 95% confidence limits.

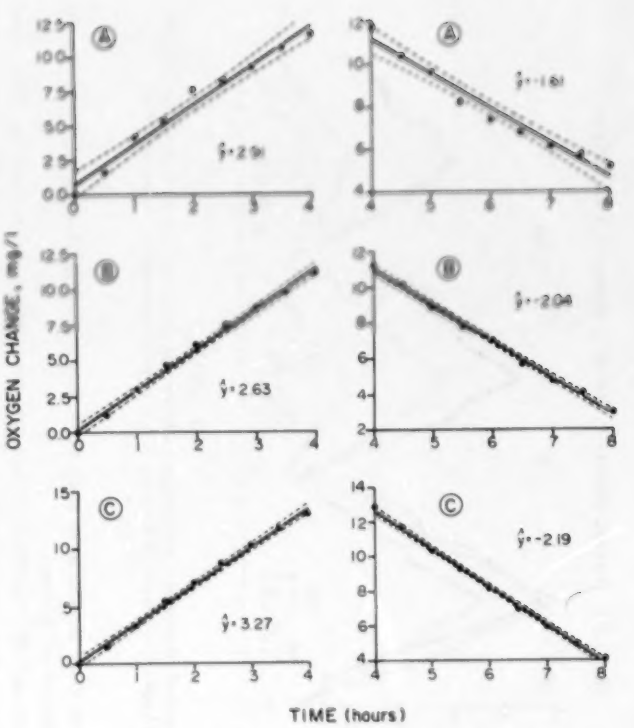

Fig. 5. Linear regression of oxygen evolution and uptake on time by *T. testudinum* in acetone control. A—pretreatment; B—40 h after treatment; C—88 h after treatment. Dashed lines represent the 95% confidence limits.

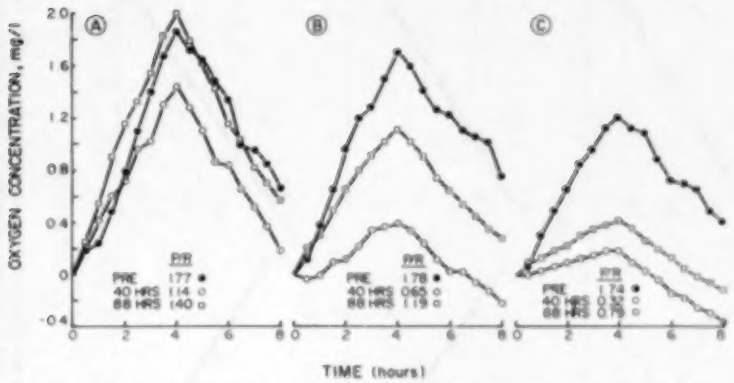

Fig. 6. Effect of atrazine on rate of oxygen evolution and uptake by *T. testudinum*. A—0·1 ppm; B—0·5 ppm; C—1·0 ppm; P/R—ratio of rate of photosynthesis to rate of respiration.

before and after treatment in each kettle and in control kettles indicated normal functioning of all systems.

Atrazine and PCP inhibited production of oxygen by *T. testudinum* (Figs 6 and 7). P/R ratios were depressed below 1 by 1 ppm of both toxicants and by 0·5 ppm atrazine (Table 1). Regression analysis (Neter & Wasserman, 1974) (Figs 8 and 9) revealed that, whereas the rate of oxygen evolution was depressed strongly after 40 h

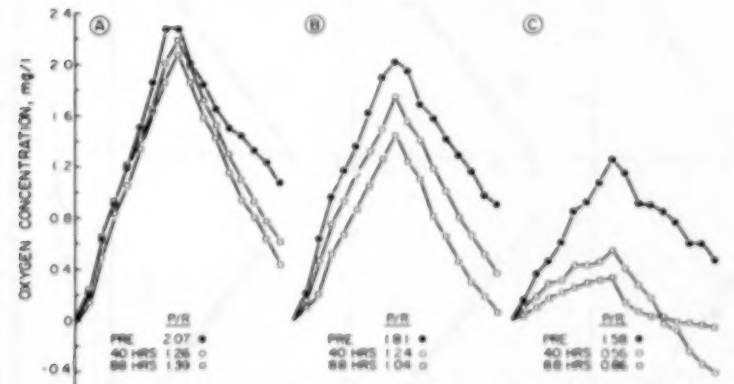

Fig. 7. Effect of PCP on rates of oxygen evolution and uptake by *T. testudinum*. A—0·01 ppm; B—0·1 ppm; C—1·0 ppm; P/R—ratio of rate of photosynthesis to rate of respiration.

TABLE 1
SUMMARY OF EFFECTS OF ATRAZINE AND PCP ON P/R RATIO OF *T. testudinum*: PRETREATMENT AND 40 AND 88 h AFTER TREATMENT

	Atrazine			*PCP*		
	Pre	*40 h*	*88 h*	*Pre*	*40 h*	*88 h*
Seawater control	1·67	1·95	1·57	1·70	1·21	1·47
Acetone control	1·62	1·46	1·61	1·81	1·37	1·49
0·01 ppm	ND	ND	ND	2·07	1·26	1·39
0·1 ppm	1·77	1·14	1·40	1·81	1·24	1·04
0·5 ppm	1·78	0·65	1·19	ND	ND	ND
1 ppm	1·74	0·32	0·79	1·58	0·56	0·86

ND = not done.

by 1 ppm of either toxicant, rate of oxygen uptake was depressed slightly by atrazine and strongly by PCP.

For comparative purposes, the test can be used to generate EC_{50} values (calculated concentrations that would inhibit oxygen evolution by 50% of the control values). In the test described above, data after 40 hours' exposure to atrazine were:

Atrazine (ppm)	*% Decrease*
0·1	15·8
0·5	77·2

Using straight-line graphical interpolation (APHA, 1975), the EC_{50} for atrazine is 0·32 ppm. This is higher than the EC_{50}'s (96-h) reported for growth of marine

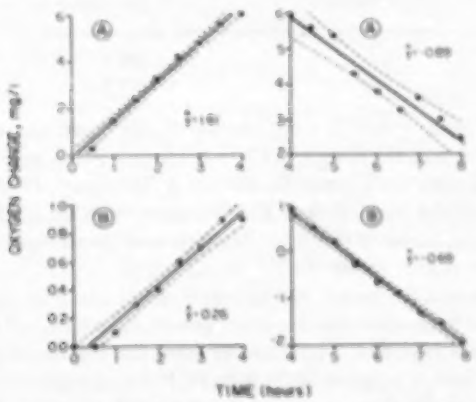

Fig. 8. Linear regression of oxygen evolution and uptake on time by *T. testudinum* treated with 1·0 ppm atrazine. A—pretreatment; B—40 h after treatment. Dashed lines represent the 95% confidence limits.

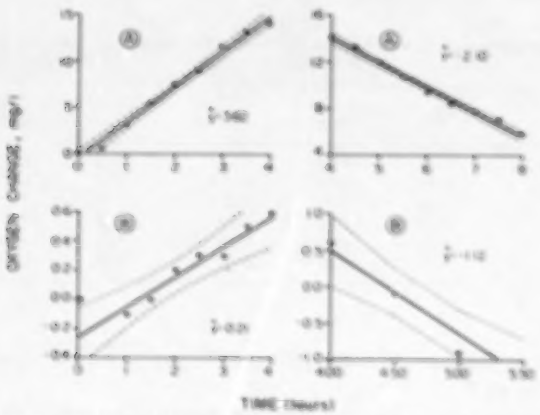

Fig. 9. Linear regression of oxygen evolution and uptake on time by *T. testudinum* treated with 1·0 ppm PCP. A—pretreatment; B—40 h after treatment. Dashed lines represent the 95% confidence limits.

unicellular algal (Walsh, 1972): *Chlorococcum* sp. (0·10 ppm); *Dunaliella tertiolecta* (0·30 ppm); *Isochrysis galbana* (0·10 ppm) and *Porphyridium cruentum* (0·20 ppm), but lower than the LC_{50}'s for estuarine animals such as *Mysidopsis bahia* (0·92 ppm); *Penaeus duorarum* (6·9 ppm); *Palaemonetes pugio* (9·0 ppm); *Uca pugilator* (29 ppm); *Crassostrea virginica* (30 ppm); *Leiostomus xanthurus* (8·5 ppm) and *Cyprinodon variegatus* (16 ppm) (Parrish *et al.*, 1982).

For the test with PCP described above, where:

PCP (ppm)	*% Decrease*
0·01	10·4
1·0	55·5

the EC_{50} is 0·74 ppm, which is only slightly higher than the LC_{50} (96-h) for *P. pugio* (0·65 ppm) and *C. variegatus* (0·33 ppm) and much higher than that for *Lagondon rhomboides* (0·07 ppm) (Borthwick & Schimmel, 1978). Marine algae were also more sensitive to PCP than *T. testudinum*; 96-h EC_{50}'s for three species are: *Skeletonema costatum* (0·02 ppm), *Thalassiosira pseudonana* (0·18 ppm) and *D. tertiolecta* (0·28 ppm) (Walsh, G. E., unpublished).

The data suggest that the leaves may recover from, or adapt to, an initial stress by atrazine or PCP. In both cases, the rate of oxygen evolution after 88 hours' exposure was greater than that after 40 h (Figs 7 and 8). Also, the P/R ratios of leaves treated with all concentrations of atrazine and 1 ppm PCP were greater at 88 h than at 40 h.

Our test can be used to measure the potential hazard of water-soluble pollutants to seagrass. Inhibition of oxygen evolution occurred soon after exposure and

although rates of oxygen flux varied from test to test, presumably because of physiological differences in the samples, responses in different tests were the same when estimated by P/R ratios and EC_{50} values. The method should be adaptable to other marine and freshwater plants.

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